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# 5-Hydroxytryptamine type 2A receptors regulate cyclic AMP accumulation in a neuronal cell line by protein kinase C-dependent and calcium/calmodulin-dependent mechanisms.

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The effects of 5-hydroxytryptamine (5-HT)2A receptor activation on cAMP formation were studied in a cell line derived from embryonic rat cortex (A1A1). 5-HT (EC50 = 0.87 microM) amplified the amount of cAMP formed in response to 5'-N-ethylcarboxamidoadenosine (an adenosine A2 receptor agonist), cholera toxin, and forskolin after 15 min of coincubation in the presence of the phosphodiesterase inhibitor rolipram. This effect of 5-HT was blocked by 10 nM ketanserin as well as by 10 nM spiperone, indicating a response mediated by the 5-HT2A receptor subtype. Similarly, cAMP accumulation was enhanced by coincubation with the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA) and the calcium ionophore A23187. After exposure to PMA for 24 hr (PKC-depleted cells), 5-HT and A23187 still enhanced cAMP formed in response to forskolin and 5'-N-ethylcarboxamidoadenosine, whereas the amplifying effects of PMA were abolished. Analysis by Western blots and PKC activity measurements revealed that, of three PKC isoforms detected in A1A1 cells (alpha, delta, and epsilon), only the calcium-independent isoform PKC-epsilon remained in membrane fractions after long term PMA treatment. In PKC-depleted cells, 5-HT-mediated amplification was greatly reduced after treatment with the calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (acetoxymethyl)-ester or the calmodulin antagonists calmidazolium and N-(6-aminohexyl)-5-chloro-1-napthalenesulfonamide hydrochloride. In addition, 5-HT-mediated amplification of cAMP accumulation was reduced by the PKC inhibitor staurosporine in normal cells but was unaffected in PKC-depleted cells. In conclusion, these data suggest that 5-HT2A receptor activation can amplify cAMP formation in A1A1 cells by two distinct pathways coupled to the hydrolysis of inositol phosphates, i.e., PKC and